

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 16-18, 20, 22, 26, and 32-36, as set forth below.

Please cancel claims 3, 4, 12-15, and 27-30.

Please withdraw claims 31 and 37, without prejudice or disclaimer.

The current listing of claims replaces all prior listings.

1. (Currently amended) A method of treating critical limb ischemia (CLI) comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein the eNOS ~~polypeptide~~comprises as polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1, and wherein the eNOS polypeptide has increased eNOS activity, as compared to wild-type eNOS polypeptide.
2. (Original) The method according to Claim 1, wherein said eNOS polypeptide is a human eNOS polypeptide.
- 3-4. (Canceled)
5. (Previously presented) The method according to Claim 1, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1.
6. (Previously presented) The method according to Claim 1, wherein said eNOS polypeptide further comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.
7. (Previously presented) The method according to Claim 1, wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495 and a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.

8. (Previously presented) The method according to Claim 1, wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495, a second mutation at a position corresponding to amino acid 1177, and a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1.

9. (Previously presented) The method according to Claim 5, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 495 is an amino acid substitution to Ala, Val, Leu, or Ile.

10. (Original) The method according to Claim 6, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 1177 is an amino acid substitution to Asp.

11. (Original) The method according to Claim 8, wherein said mutation at a position corresponding to amino acid residue 2 is an amino acid substitution to Ala.

12-15. (Canceled)

16. (Currently amended) The method according to Claim 1[[5]], wherein said activity is the generation of NO.

17. (Currently amended) The method according to Claim 1[[5]], wherein said activity is reductase activity.

18. (Currently Amended) The method according to Claim 1[[2]], ~~13, 14, 15~~, 16, or 17, wherein the amino acid sequence of said reference wild-type polypeptide is the amino acid sequence of a human eNOS.

19. (Previously presented) The method according to Claim 18, wherein the amino acid sequence of said wild-type polypeptide is SEQ ID NO: 1.
20. (Currently amended) The method according to Claim ~~[[1]]~~ 35 or 36, wherein the amino acid sequence of said eNOS polypeptide comprises mutations at amino acid residues corresponding to threonine 495 and serine 1177 of a human eNOS comprising the amino acid sequence of SEQ ID NO:1.
21. (Original) The method according to Claim 20, wherein the amino acid sequence of said eNOS polypeptide has a 95-99 % sequence identity to the amino acid sequence of SEQ ID NO: 1.
22. (Currently Amended) The method according to Claim 1 ~~or 12~~, wherein said polynucleotide is a recombinant vector comprising a nucleic acid sequence encoding said eNOS polypeptide and said sequence is operably linked to at least one regulatory sequence such that said polypeptide is expressed in cells.
23. (Original) The method according to Claim 22, wherein said nucleic acid sequence is operably linked to a promoter.
24. (Original)The method according to Claim 23, wherein said recombinant vector is a viral vector.
25. (Original) The method according to Claim 24, wherein said viral vector is an adenoviral vector.
26. (Currently amended) The method according to Claim 1 ~~or 12~~, wherein said treating comprises modulating eNOS activity in cells of said patient.

27-30. (Canceled)

31. (Withdrawn) The method according to Claim 1 or 4, wherein said administering comprises introducing said polynucleotide to cells of said patient ex vivo.

32. (Currently amended) The method according to Claim 1 ~~or 12~~, wherein said administering comprises delivery of said polynucleotide to a diseased tissue of said patient.

33. (Currently amended) The method according to Claim 1 ~~or 12~~, wherein said administering comprises delivery of said polynucleotide to the peripheral vascular system of said patient.

34. (Previously presented) The method according to Claim 33, wherein said delivery is to a limb muscle of said patient.

35. (Currently amended) A method of treating an angiogenesis-dependent disorder comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1, and wherein the eNOS polypeptide has increased eNOS activity, as compared to wild-type eNOS polypeptide.

36. (Currently amended) A method of ameliorating microvascular dysfunction comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino

acid residues 478-522 of SEQ ID NO:1, and wherein the eNOS polypeptide has increased eNOS activity, as compared to a wild-type eNOS polypeptide.

37. (Withdrawn) A method of treating critical limb ischemia (CLI) comprising administering to a patient in need of treatment an effective amount of an eNOS polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a mammalian eNOS that is phosphorylated in mammalian cells.

38. (Previously presented) The method according to Claim 35 or 36, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Ala, Val, Leu, or Ile.

39. (Previously presented) The method according to Claim 35 or 36, wherein said eNOS polypeptide further comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Asp.

40. (Previously presented) The method according to Claim 35 or 36, wherein said eNOS polypeptide comprises:

- i) a first mutation at a position corresponding to amino acid 495 of SEQ ID NO:1, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile; and
- ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp.

41. (Previously presented) The method according to Claim 35 or 36, wherein said eNOS polypeptide comprises:

- i) a first mutation at a position corresponding to amino acid 495 of SEQ ID NO:1, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile;
- ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp; and

iii) a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Ala.

42. (Previously presented) The method according to any of Claims 9, 38, 40, or 41, wherein said mutation at a position corresponding to amino acid residue 495 is an amino acid substitution to Val.